KNIGHT CANCER INSTITUTE
Oregon Health & Science University

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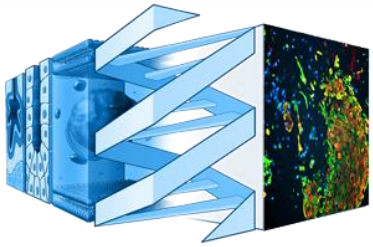
Knight Cancer Institute



OHSU CENTER FOR SPATIAL SYSTEMS BIOMEDICINE

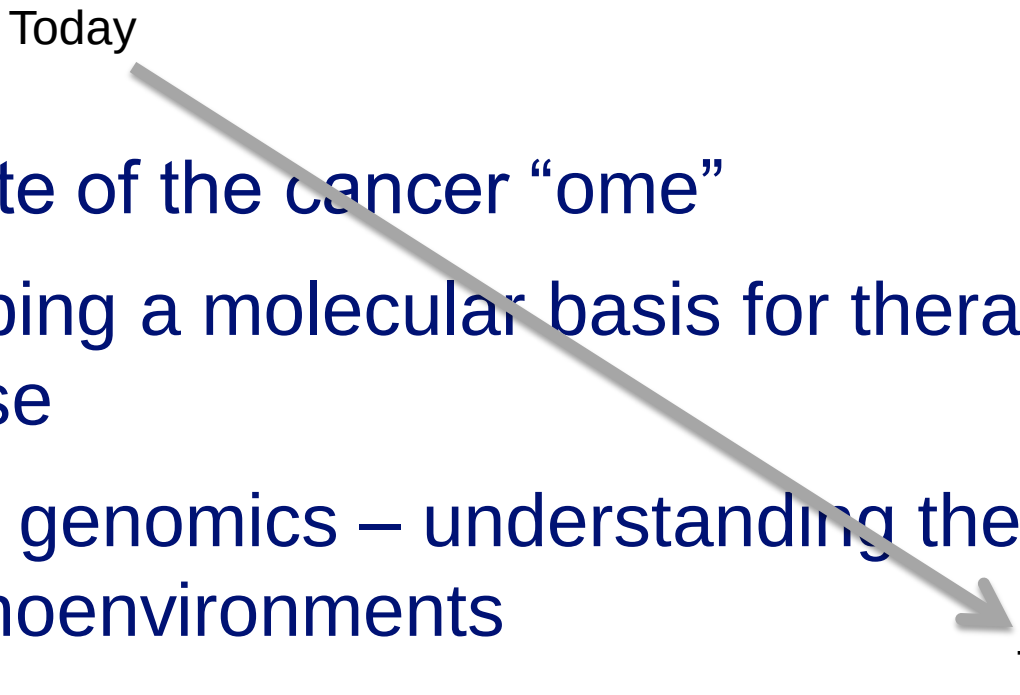
Predicting responses to cancer therapies

Omics and beyond

KAVLI INSTITUTE FOR THEORETICAL PHYSICS
May 24, 2012

Omics and beyond

Topics for discussion



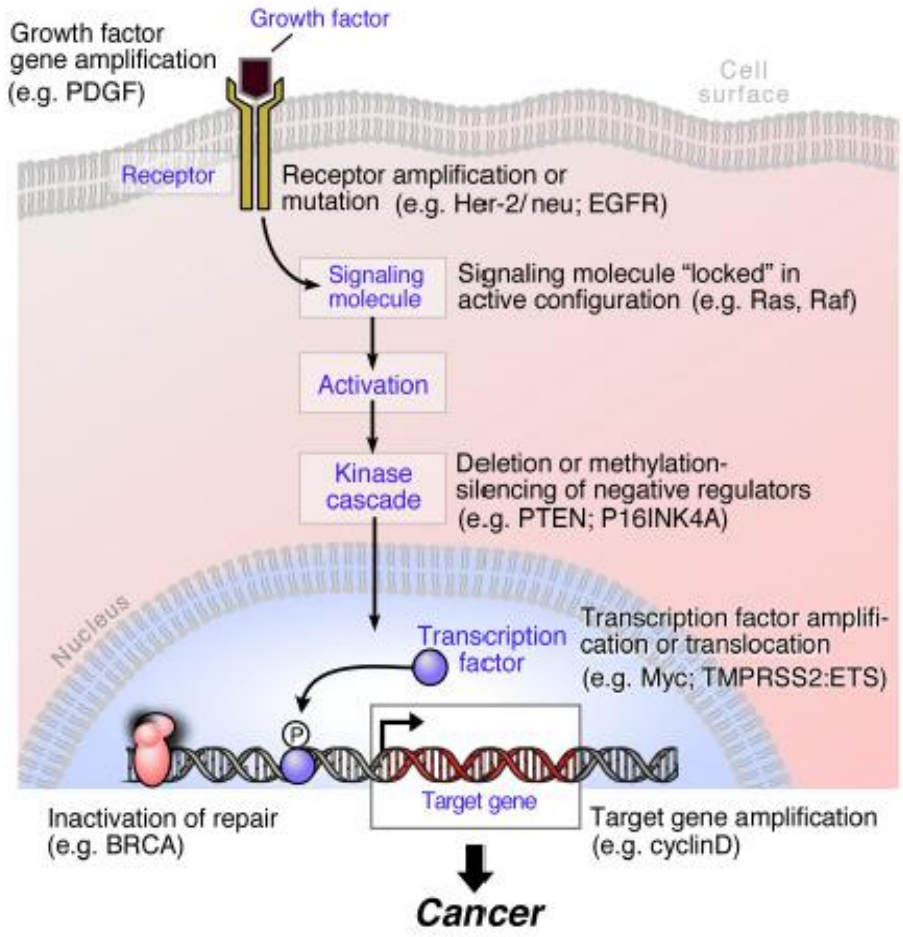
- The state of the cancer “ome”
- Developing a molecular basis for therapeutic response
- Beyond genomics – understanding the micro- and nanoenvironments

Omics and beyond

Topics for discussion

- The state of the cancer “ome”
- Developing a molecular basis for therapeutic response
- Beyond genomics – understanding the micro- and nanoenvironments

Goals in cancer research



- Normal cells have established regulatory systems to control behavior (grow, die, move, secrete a protein, etc.)
- Cancers arise because of genetic damage
- Our goal in cancer treatment is to find the abnormalities and develop therapies to correct the defect

Investments in genome science have stimulated development of a wealth of “reductionist” tools to catalog omic components

Massively parallel DNA

Microarrays

Microfluidics and PCR

LC/mass spec

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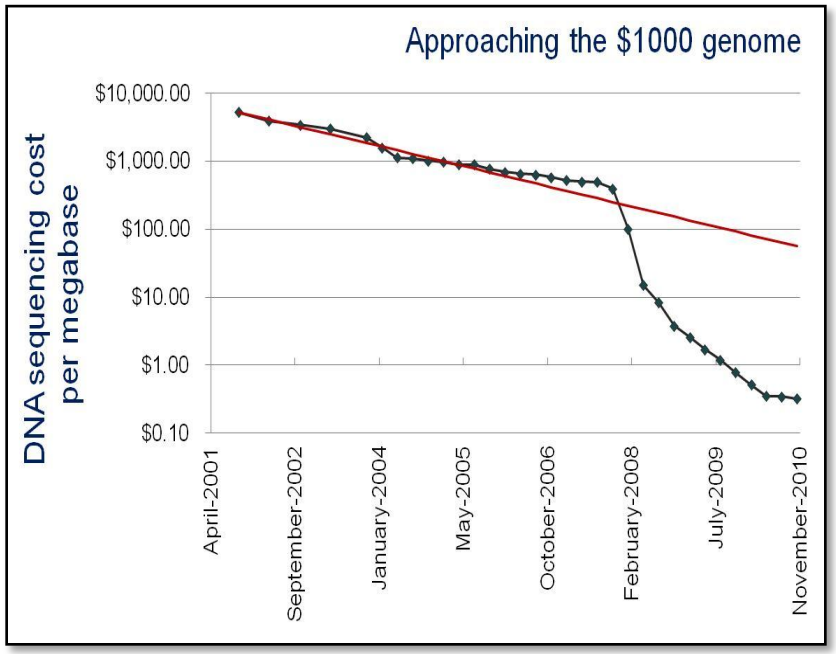
Plummeting costs and technological advances make clinical applications increasingly practical

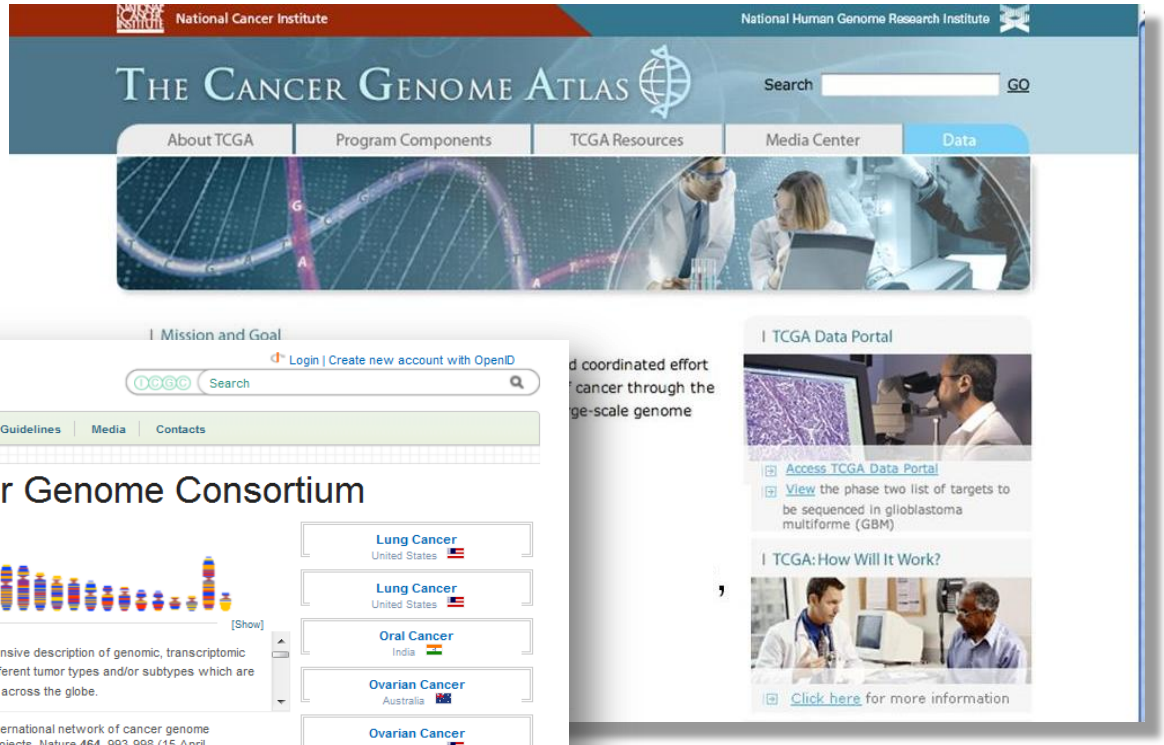
Nucleic acid sequencing



- Microgram to nanogram quantities of nucleic acids for copy number, mutation and promoter methylation
- Formalin fixed paraffin embedded capability
- Genome wide analysis ~$1500/sample
- Real time (~1 hour), point of care diagnostics

International cancer genomics efforts are cataloging important genome aberrations in major tumor types

Recurrent aberrations will be defined for major tumor types in 3 -5 years

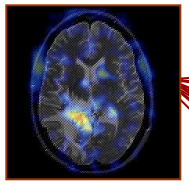


Copy number, mutations, structural changes, expression, splicing, promoter methylation, miRNAs, protein levels

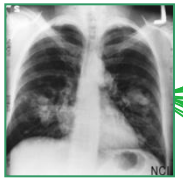
TCGA: A comprehensive approach to aberrant pathway definition

25 forms of cancer

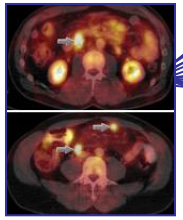
glioblastoma multiforme (brain)



squamous carcinoma (lung)



serous cystadenocarcinoma (ovarian)



Etc. Etc. Etc.

Biospecimen Core Resource with more than 150 Tissue Source Sites

6 Cancer Genomic Characterization Centers

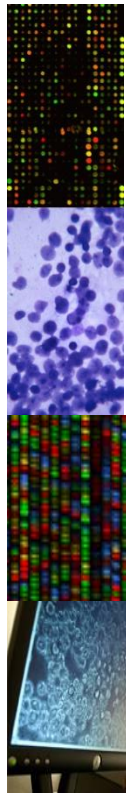
3 Genome Sequencing Centers

7 Genome Data Analysis Centers

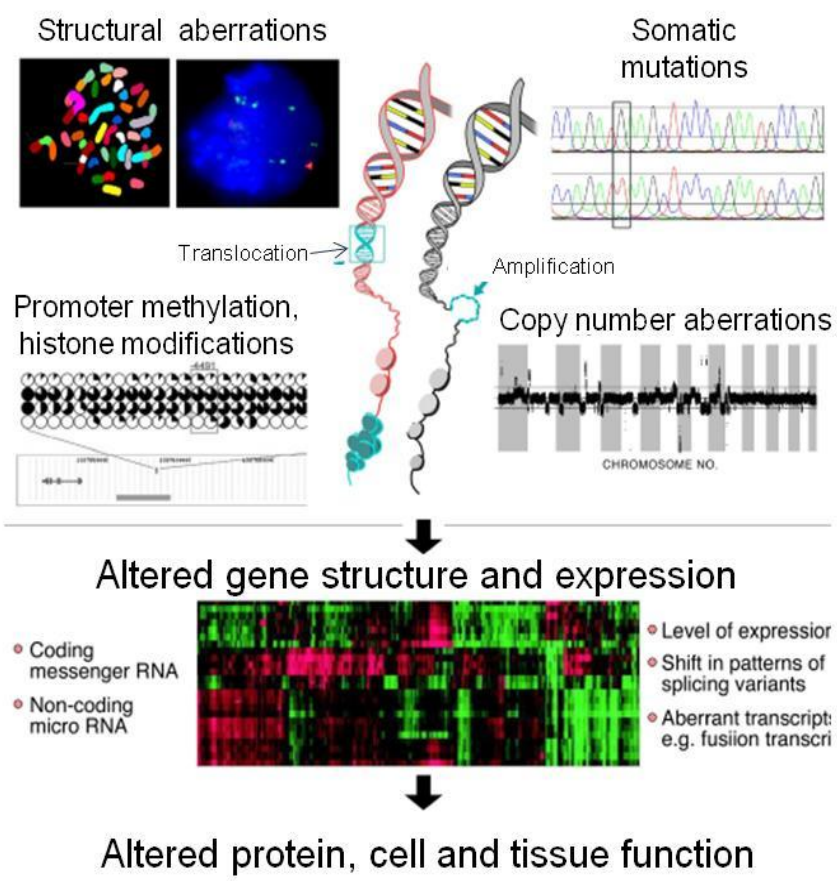
Data Coordinating Center

Multiple data types

- Clinical diagnosis
- Treatment history
- Histologic diagnosis
- Pathologic report/images
- Tissue anatomic site
- Surgical history
- Gene expression/RNA sequence
- Chromosomal copy number
- Loss of heterozygosity
- Methylation patterns
- miRNA expression
- DNA sequence
- RPPA (protein)
- Subset for Mass Spec



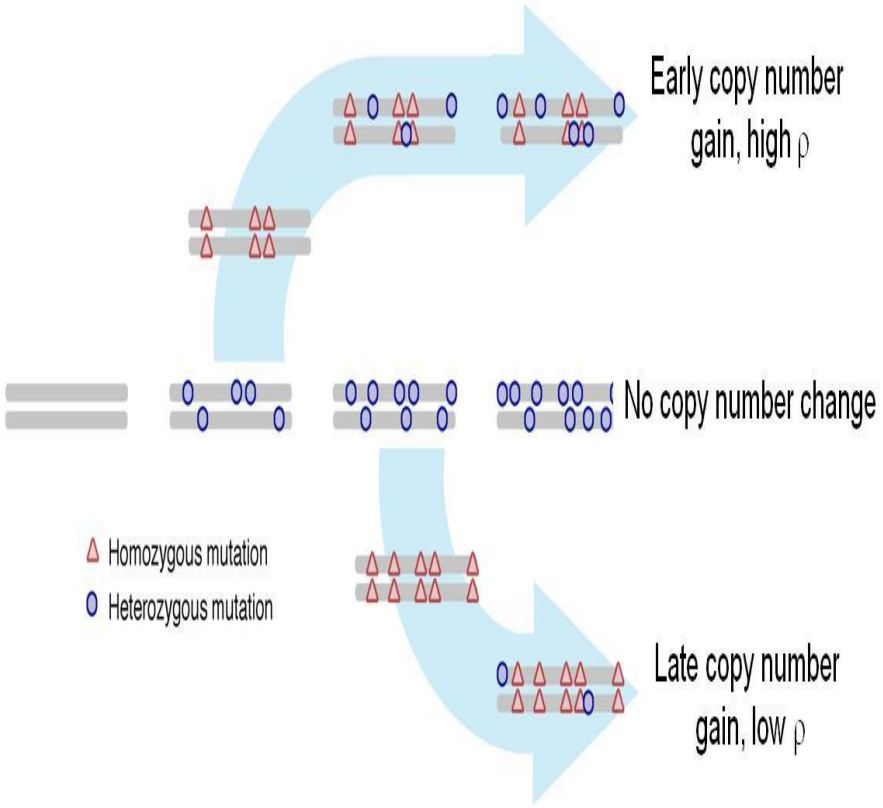
State of the cancer genome



- Multiple genomic mechanisms deregulate genes that contribute to cancer pathophysiology
- Some aberrations occur earlier than others and can be ordered using information from one sample
- Recurrent aberration patterns define breast cancer subtypes that differ in outcome and therapeutic response

Chin & Gray, Nature 2010

State of the cancer genome



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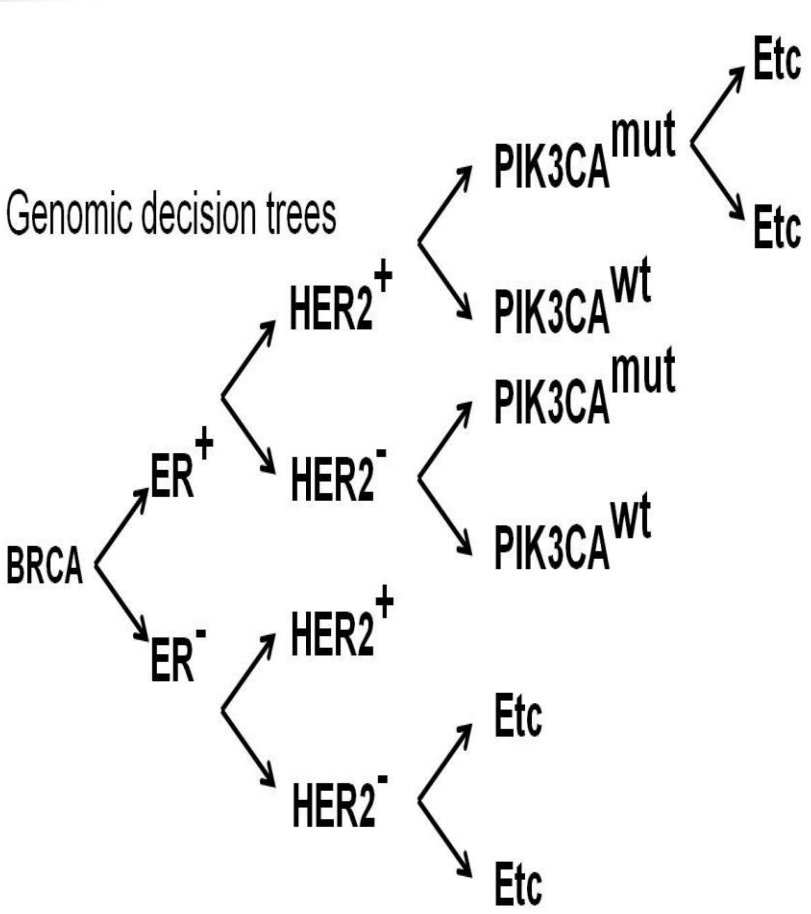
Durinck , Cho et al, Cancer Discovery, 2011

State of the cancer genome



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Omics and beyond

Topics for discussion

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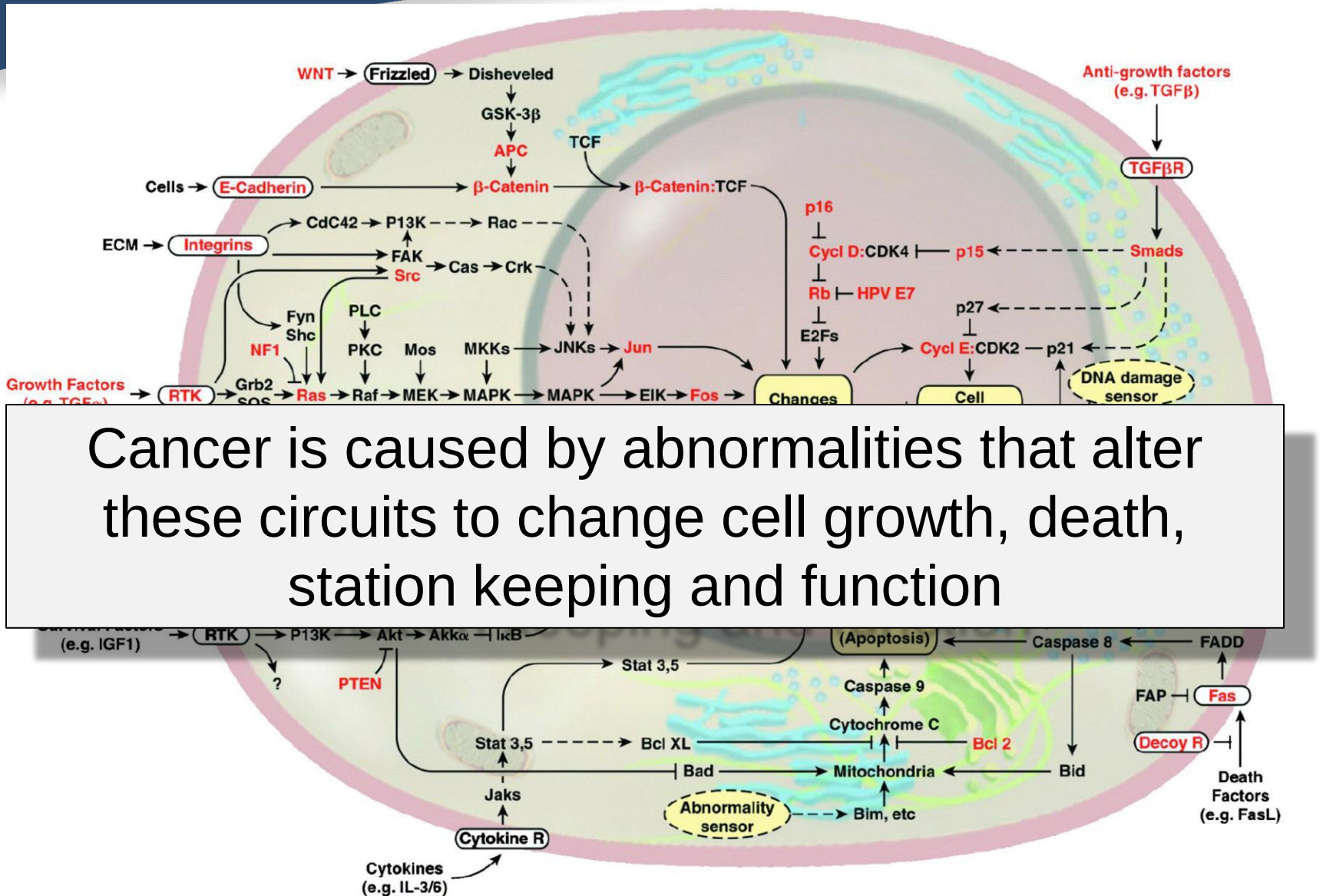
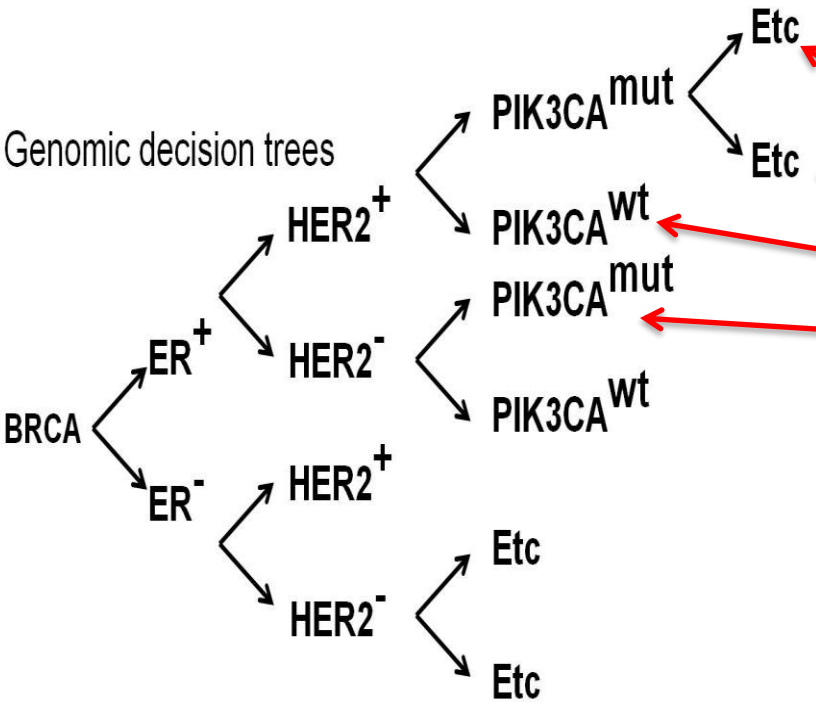
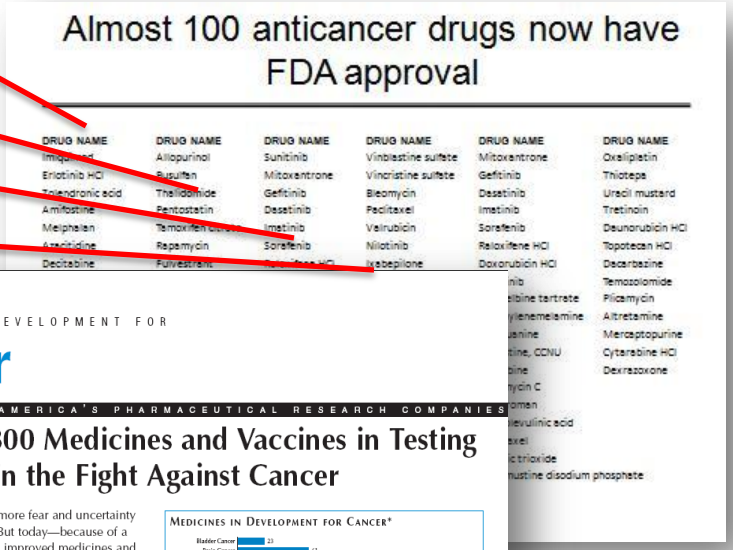
Genomic aberrations deregulate cellular regulatory systems



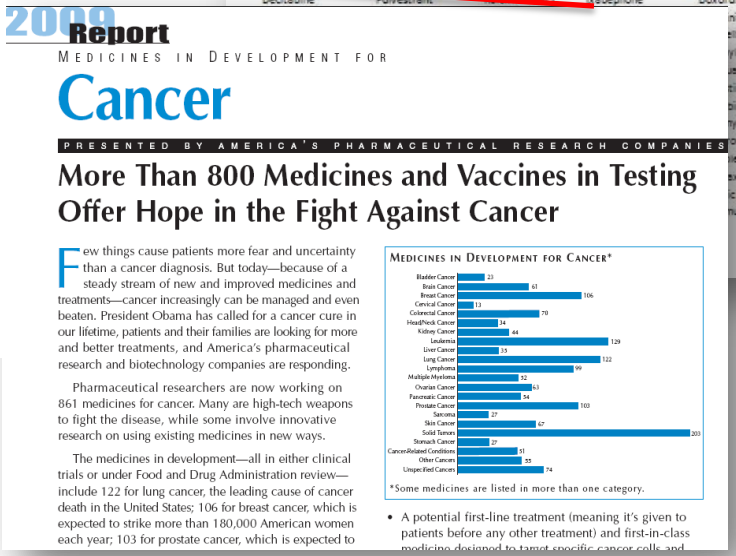
Therapeutic approaches either attempt to restore proper regulation or inhibit growth of deregulated cells

>$100 Billion in drug development




Almost 100 anticancer drugs now have FDA approval

DRUG NAME	DRUG NAME	DRUG NAME	DRUG NAME	DRUG NAME	DRUG NAME
Imiquimod	Allopurinol	Sunitinib	Vinblastine sulfate	Mitoxantrone	Oxaliplatin
Erlotinib HCl	Busulfan	Mitoxantrone	Vincristine sulfate	Gefitinib	Thiotepa
Zoledronic acid	Thalidomide	Gefitinib	Bleomycin	Dasatinib	Uracil mustard
Amifostine	Pentostatin	Dasatinib	Paclitaxel	Imatinib	Tretinoin
Melphalan	Tamoxifen citrate	Imatinib	Valrubicin	Sorafenib	Daunorubicin HCl
Azacitidine	Rapamycin	Sorafenib	Nilotinib	Raloxifene HCl	Topotecan HCl
Decitabine	Fulvestrant	Lapatinib ditosylate	Ixabepilone	Doxorubicin HCl	Decarbazine
					Temozolomide
					Plicamycin
					Altretamine
					Mercaptopurine
					Cytarabine HCl
					Dexrazoxane


2009 Report
MEDICINES IN DEVELOPMENT FOR
Cancer
PRESENTED BY AMERICA'S PHARMACEUTICAL RESEARCH COMPANIES

More Than 800 Medicines and Vaccines in Testing Offer Hope in the Fight Against Cancer

Few things cause patients more fear and uncertainty than a cancer diagnosis. But today—because of a steady stream of new and improved medicines and treatments—cancer increasingly can be managed and even beaten. President Obama has called for a cancer cure in our lifetime, patients and their families are looking for more and better treatments, and America's pharmaceutical research and biotechnology companies are responding.

Pharmaceutical researchers are now working on 861 medicines for cancer. Many are high-tech weapons to fight the disease, while some involve innovative research on using existing medicines in new ways.

The medicines in development—all in either clinical trials or under Food and Drug Administration review—include 122 for lung cancer, the leading cause of cancer death in the United States; 106 for breast cancer, which is expected to strike more than 180,000 American women each year; 103 for prostate cancer, which is expected to

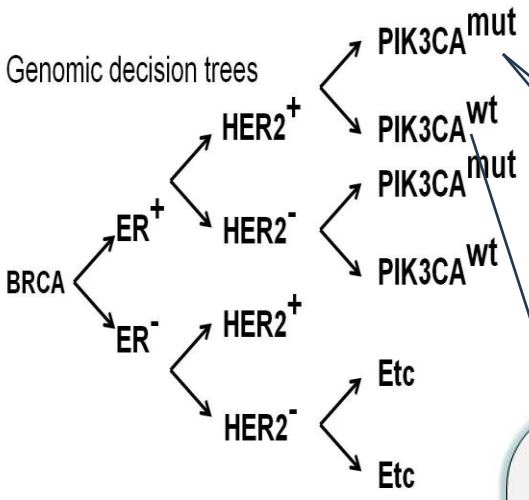
MEDICINES IN DEVELOPMENT FOR CANCER*

*Some medicines are listed in more than one category.

- A potential first-line treatment (meaning it's given to patients before any other treatment) and first-in-class

Modeling the decision tree – panels of cell lines that capture the important aberration combinations

Our goal is to include multiple cell lines in the panel that model each important decision state

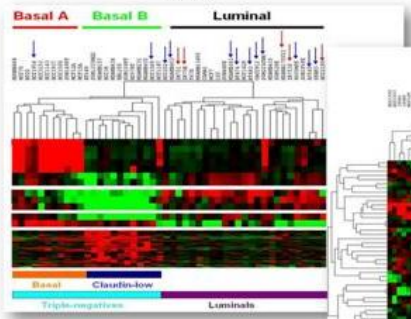


Breast cancer cell lines

184A1	HCC1143BL	HCC2218	MDAMB157	SUM1315MO2	ZR7530
184B5	HCC1187	HCC3153	MDAMB175VII	SUM149PT	ZR75B
600MPE	HCC1395	HCC38	MDAMB231	SUM159PT	
AU565	HCC1419	HCC38BL	MDAMB361	SUM185PE	
BT20	HCC1428	HCC70	MDAMB415	SUM190PT	
BT474	HCC1500	HS578T	MDAMB435	SUM225CWN	
BT483	HCC1569	LY2	MDAMB436	SUM229PE	
BT549	HCC1599	M4A4	MDAMB453	SUM44PE	
CAMA1	HCC1806	MB157	MDAMB468	SUM52PE	
DU4475	HCC1937	MCF10A	MX1	T47D	
HBL100	HCC1954	MCF10F	NM2C5	T47D_KBluc	
HCC1007	HCC202	MCF12A	S1	UACC812	
HCC1008	HCC2157	MCF7	SKBR3	UACC893	
HCC1143	HCC2185	MDAMB134VI	SUM102PT	ZR751	

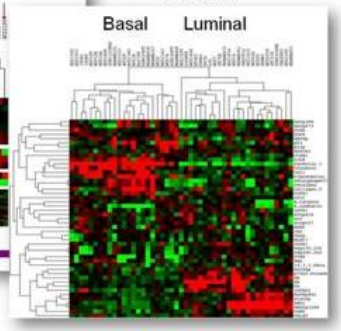
This works only to the extent that the cell lines capture the genomic events that determine response in tumors

RNA



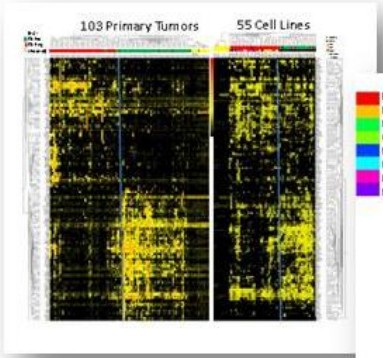
Spellman, Sadananadam

Protein



Mills, Lu

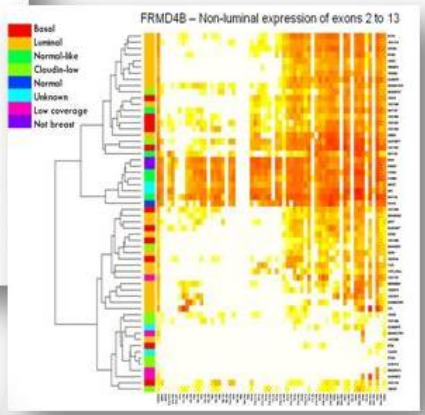
Promoter methylation



Genome copy number

Sukumar

Alternative splicing



Griffith, Daeman

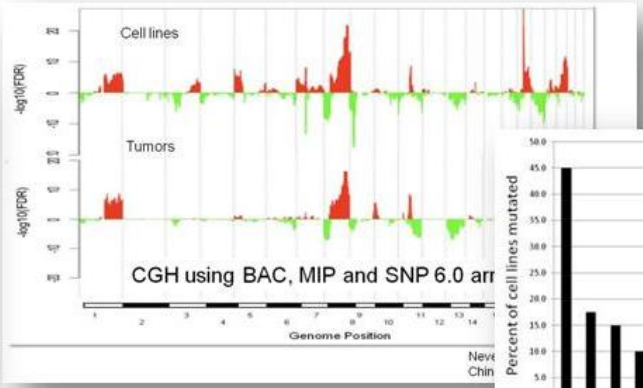
Fusion transcripts

Cell lines	Number of fusion genes
600MPE	8
BT20	21
BT474	5
BT483	1
BT459	17
HCC1143	0
HCC1149	7
HCC1428	18
HCC1500	19
HCC1569	24
HCC1599	14
HCC1806	18
HCC1937	9
HCC1954	33
HCC202	3
HCC2218	10

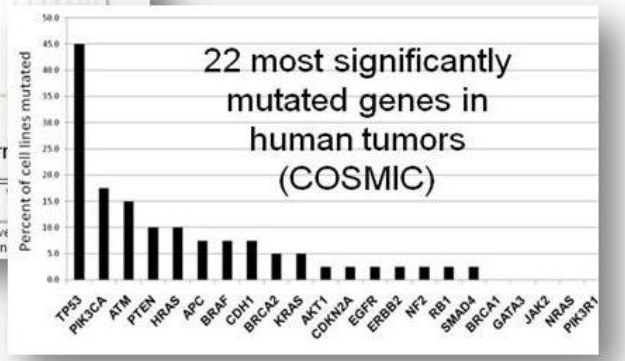
Cell lines	Number of fusion genes
HCC3153	16
HCC70	17
LY2	8
MCF7	5
MDAMB134VI	14
MDAMB361	34
MDAMB453	17
SKBR3	13
SUM149PT	1

332 total fusions
5.3/line average
ERBB2, GRB7, SMAD2 recurrent

Sadanadam



Mutations

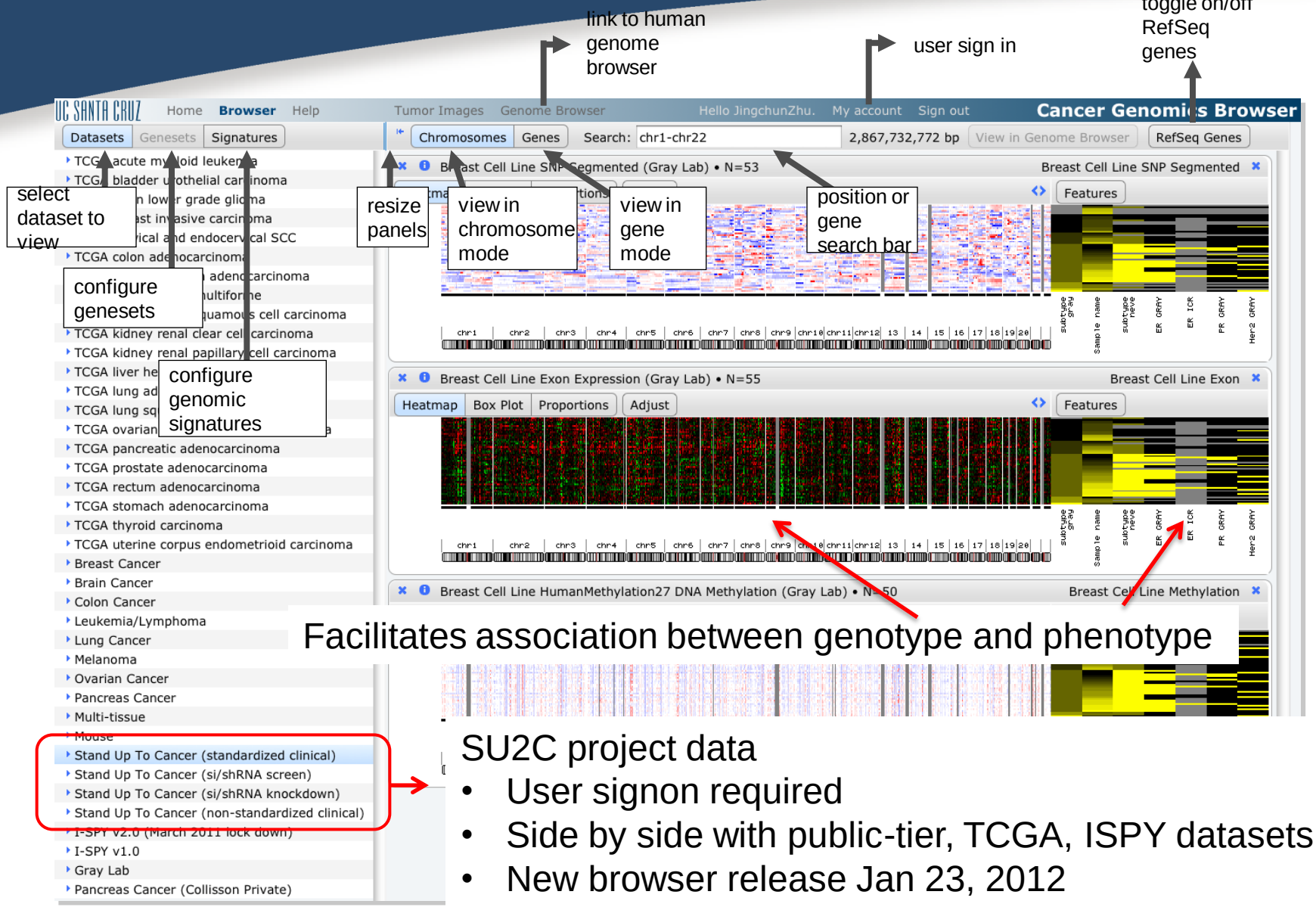


Pepin, Durinck

Kuo, Spellman, Griffith, Heiser, Chin, Sadanadam, Wang, Sukumar, Pepin, et al

The cancer genome browser

genome-cancer.ucsc.edu



Facilitates association between genotype and phenotype

SU2C project data

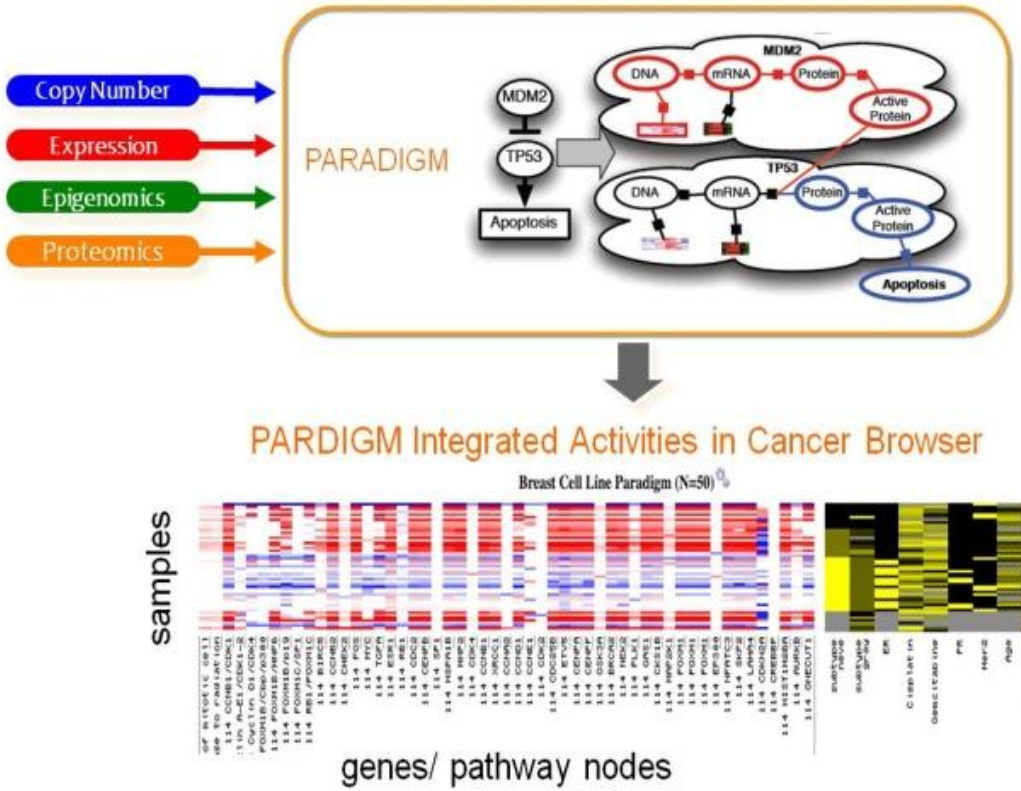
- User signon required
- Side by side with public-tier, TCGA, ISPY datasets
- New browser release Jan 23, 2012

Understanding target pathways

Cancer Genome Browser
PARADIGM activities

Stuart/Haussler et al

Informatics consortium



SU2C

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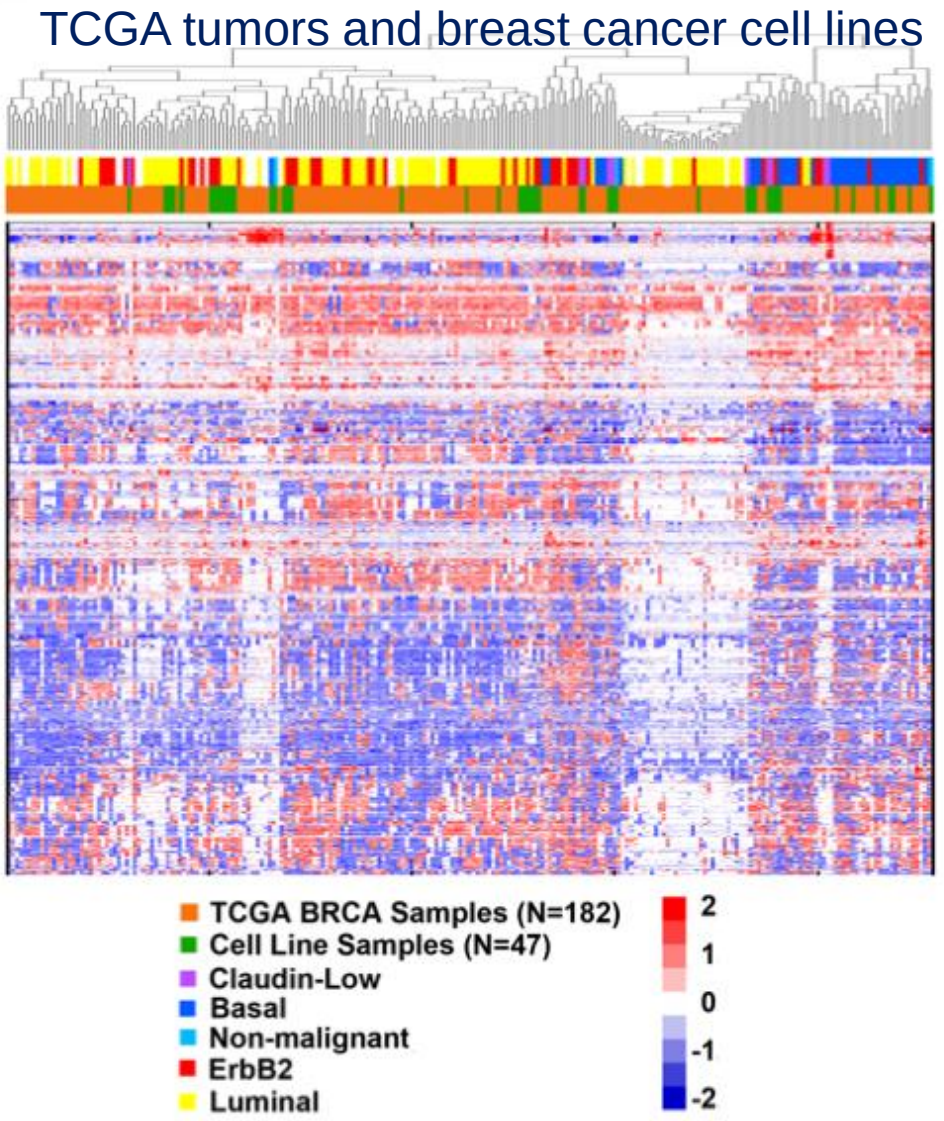
Using PARADIGM to assess subtype specific pathway

Activities for 1441 pathways

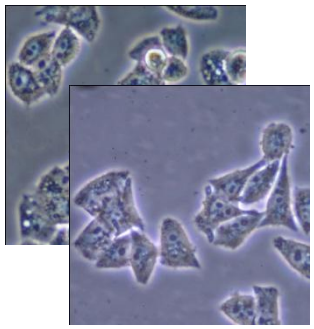
- Tumors and cell lines show common pathway signatures
- Pathway activities show strong subtype specificity



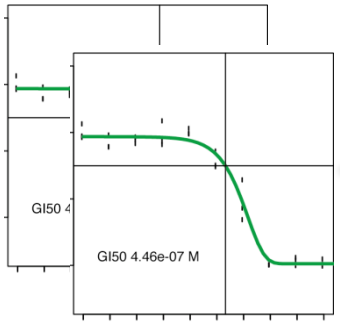
Heiser et al., PNAS 109:2724, 2012

Predictive markers are discovered by correlating the “ome” with quantitative response

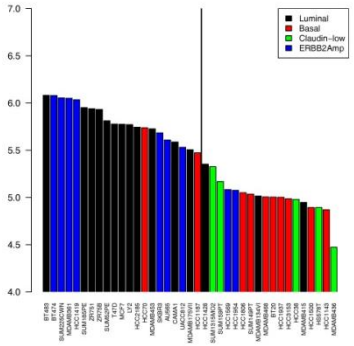
- Tested >140 therapeutic compounds in 54 cell lines
- Treat for 72 hours
- Test 9 drug doses: low to high
- 3 replicates
- GI50: Drug dose required to inhibit growth by 50%



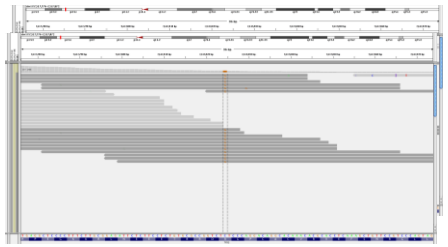
Breast cancer cell line collection

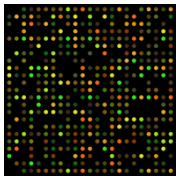


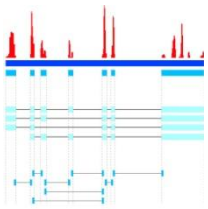
Drug/siRNA response



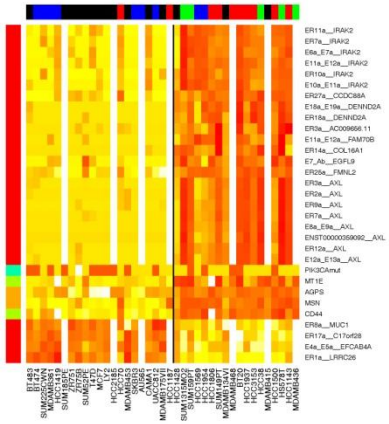
Response status







Molecular data: 9+ data types



Molecular drug response signatures

Heiser et al., PNAS 109:2724, 2012

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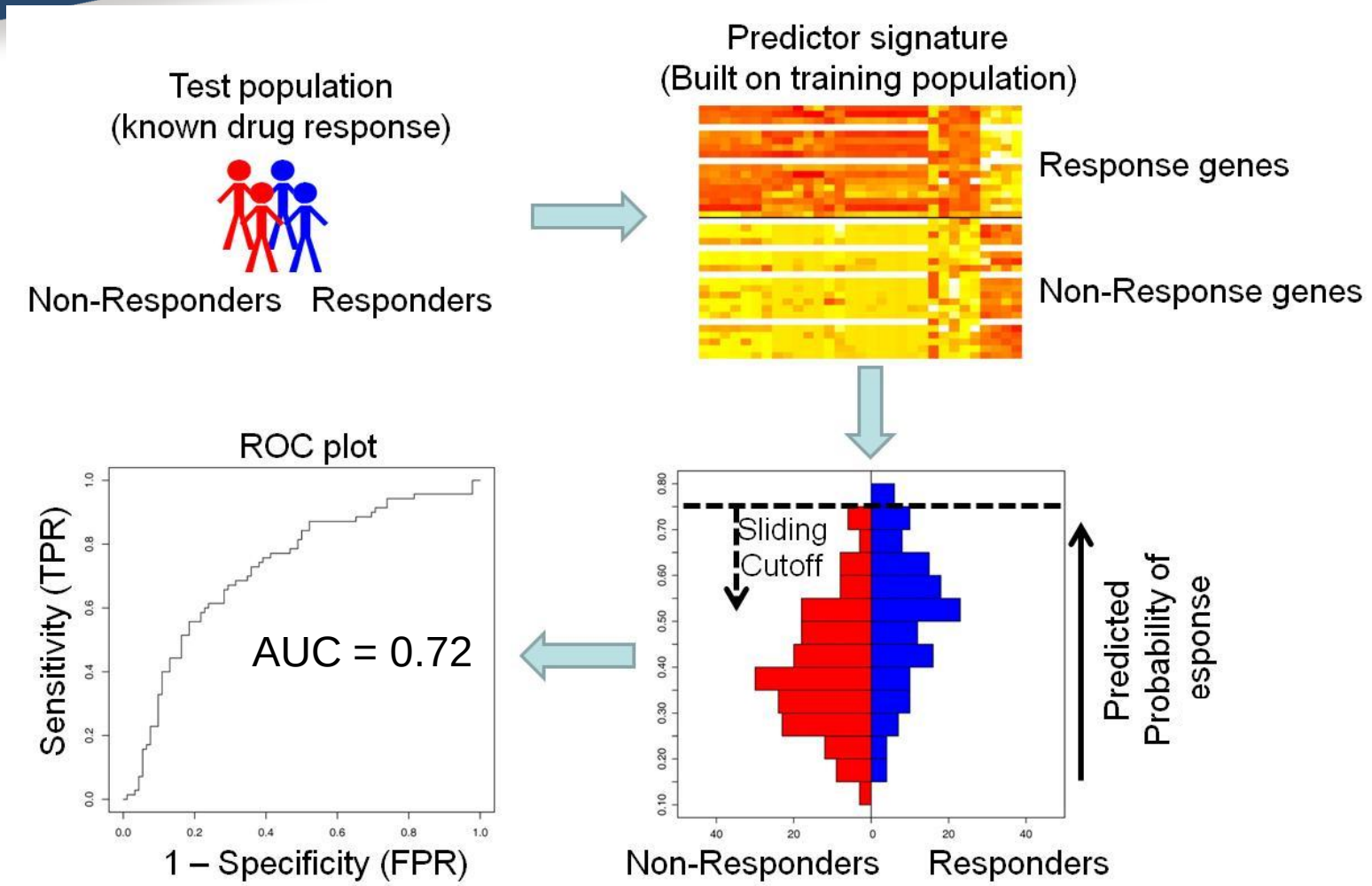
Machine learning approaches (random forests) identify quantitative predictive decision trees

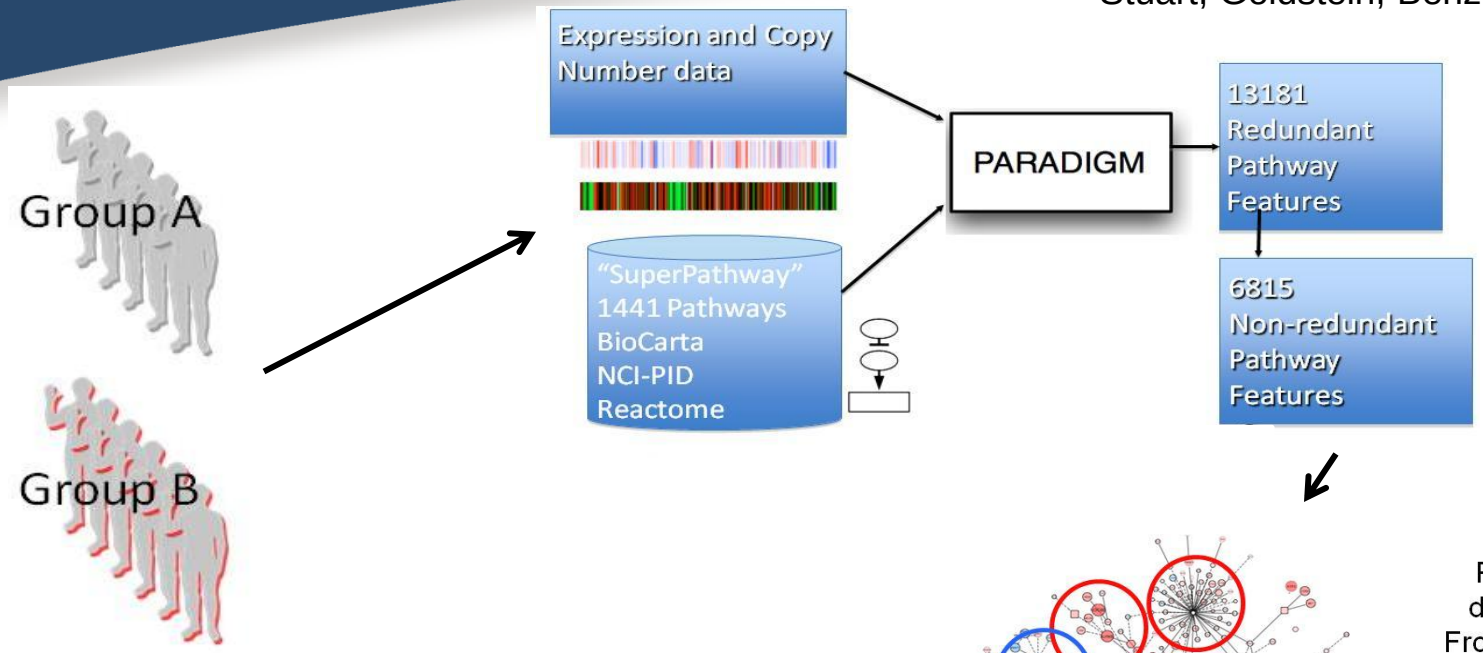


Anneleen Daemen

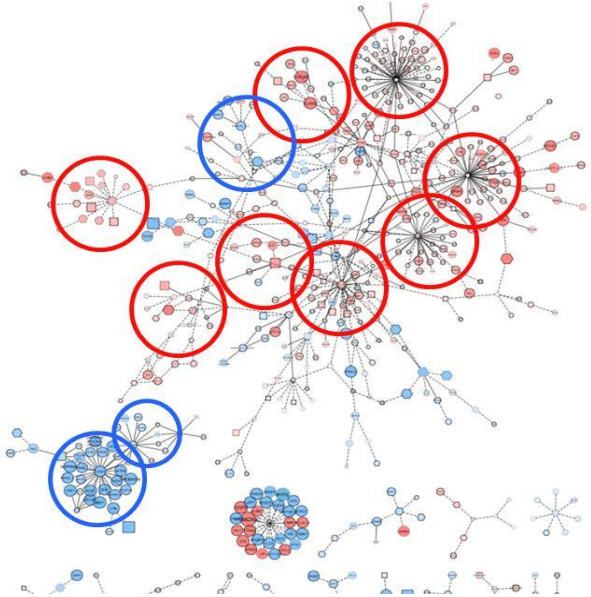
Obi Griffith

“Superpathways”– generating information on mechanisms of response

Stuart, Goldstein, Benz, Ng, Haussler



- Many small pathways are nice for understanding specific contexts but information is duplicated or incomplete when assessed independently
- Merge all small pathways using an exhaustive breadth-first search of the current database of pathways



Pathway “hubs” distinguish basal From other cell lines

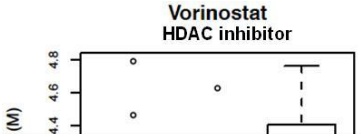
965 nodes connected by 941 edges

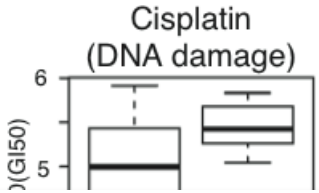
Up-regulated hub

Down-regulated hub

This approach identified pathway subnetworks associated with compound response

Lau
Hei



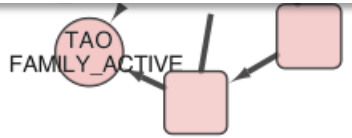


We are now applying the siRNA knockdown to validate the predicted networks

Goal: Predict molecular and phenotype response for each gene in the network and test via siRNA knockdown

Compounds attacking validated subnetworks are candidate therapeutics

Histone deacetylase network



DNA repair network

Heiser et al., PNAS 109:2724, 2012

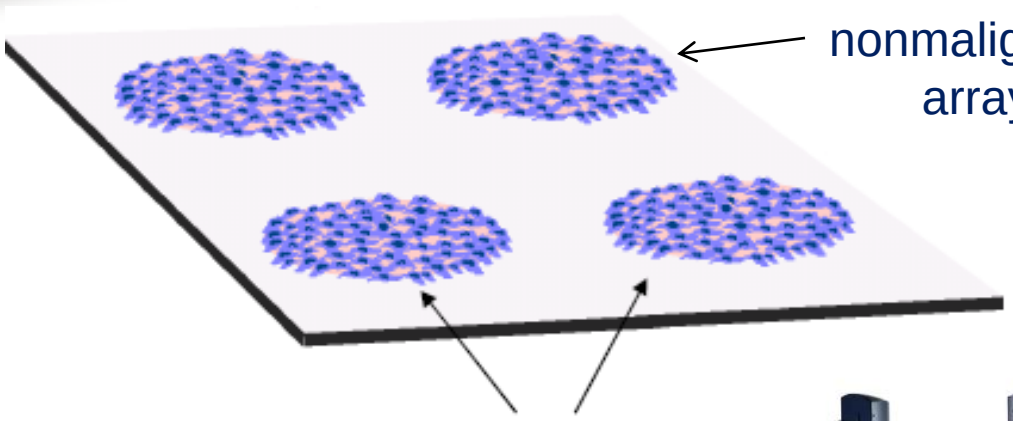
This approach identified pathway subnetworks associated with compound response

We are now applying the siRNA knockdown to validate the predicted networks

Goal: Predict molecular and phenotype response for each gene in the network and test via siRNA knockdown

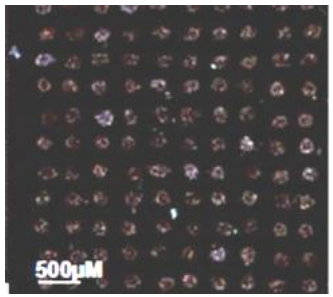
Compounds attacking validated subnetworks are candidate therapeutics

Validating “driver” pathways that affect specific cancer hallmarks

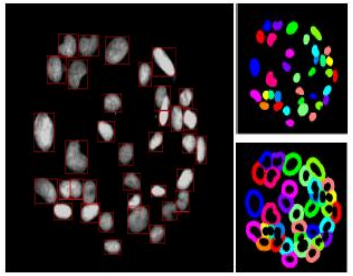


~100 malignant or nonmalignant cells per array element

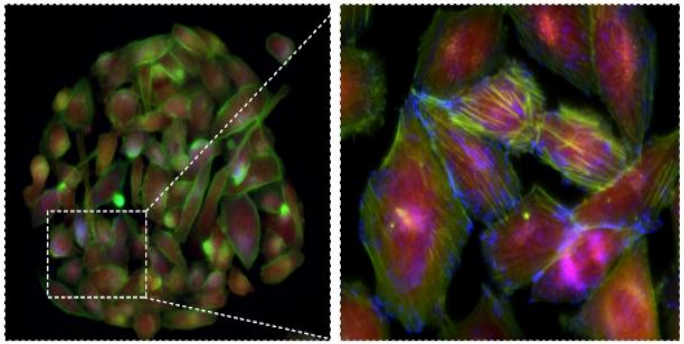
Image based assessment of IHC defined responses



Array elements contain siRNAs targeting pathways



20,000 culture elements per array

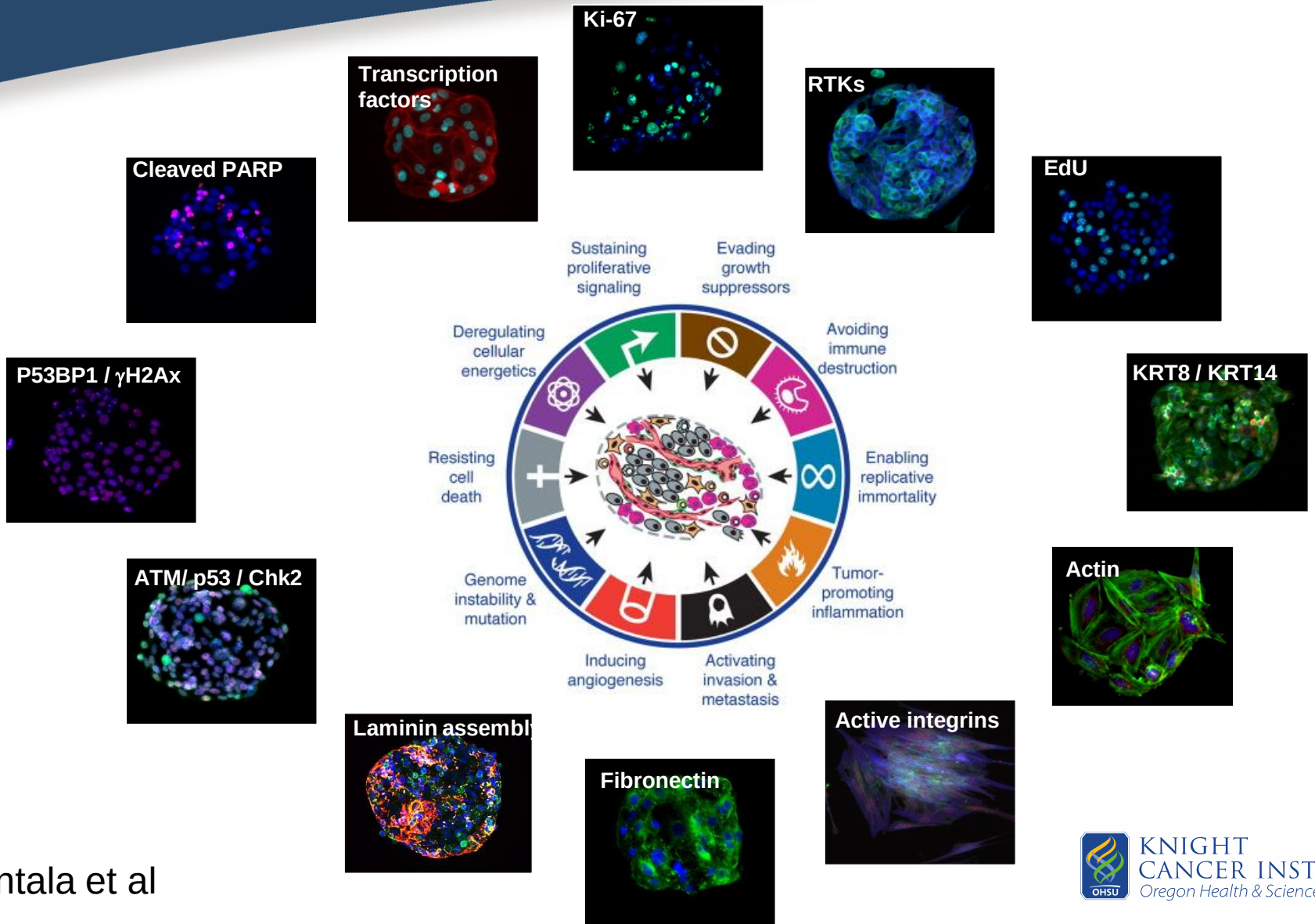


ANALYSIS OF:

- CELL COUNT
- CELL CYCLE
- IHC Ab STAINING
- ORGANELLE STAINING
- CELL MORPHOLOGY
- MIGRATION/ ADHESION
- CELL POPULATION

Rantala, Boddapati, Muschler, Hu, Kwon, …

Imaging assays for cancer hallmarks



Rantala et al

KNIGHT CANCER INSTITUTE
Oregon Health & Science University

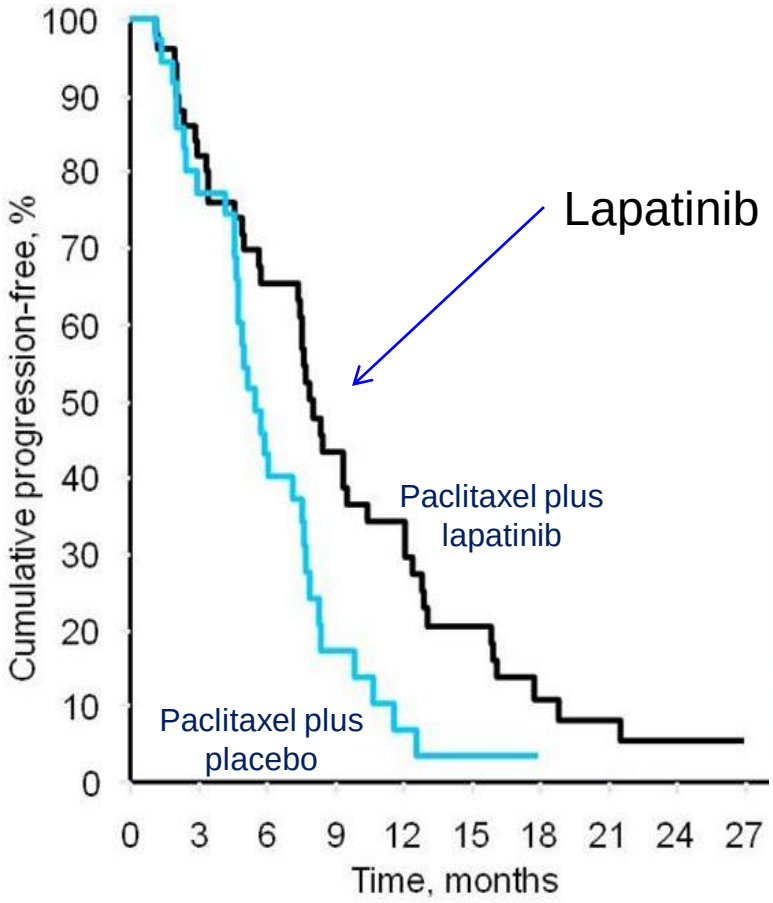
Omics and beyond

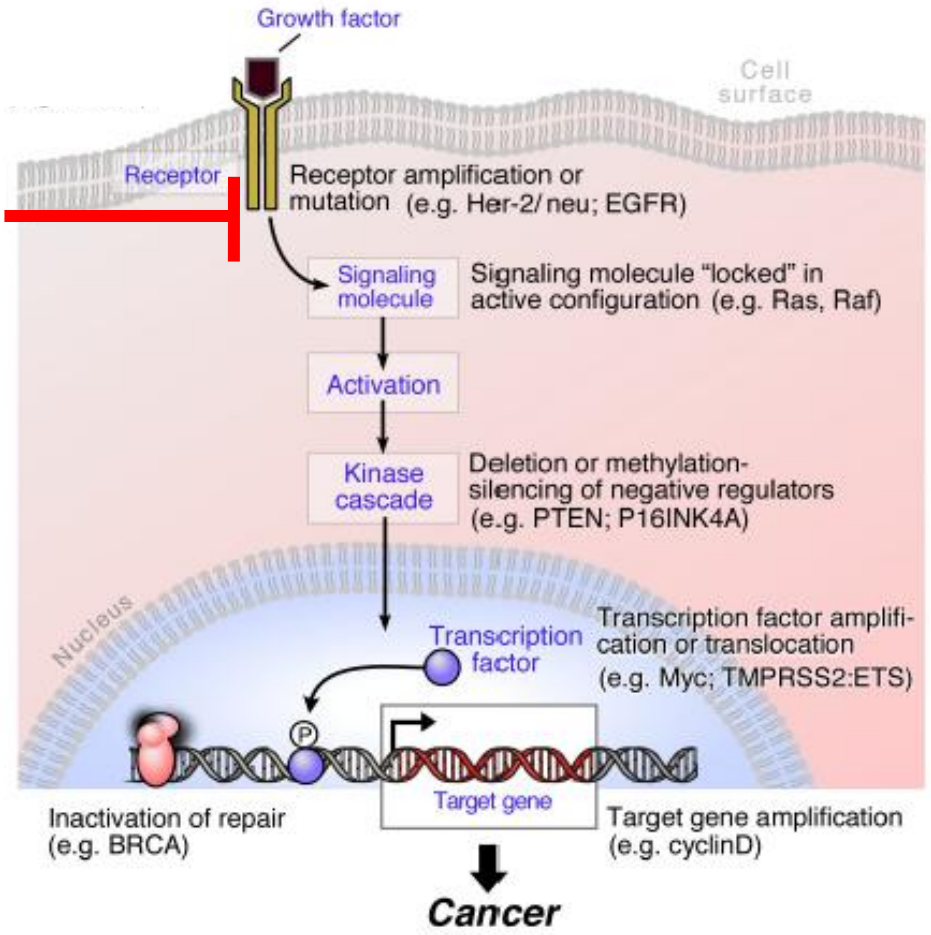
Topics for discussion

- The state of the cancer “ome”
- Developing a molecular basis for therapeutic response
- Beyond genomics – understanding the micro- and nanoenvironments

Genome aberration targeted therapies are not as durable as needed

Understanding the micro- and nano-environments of cancer

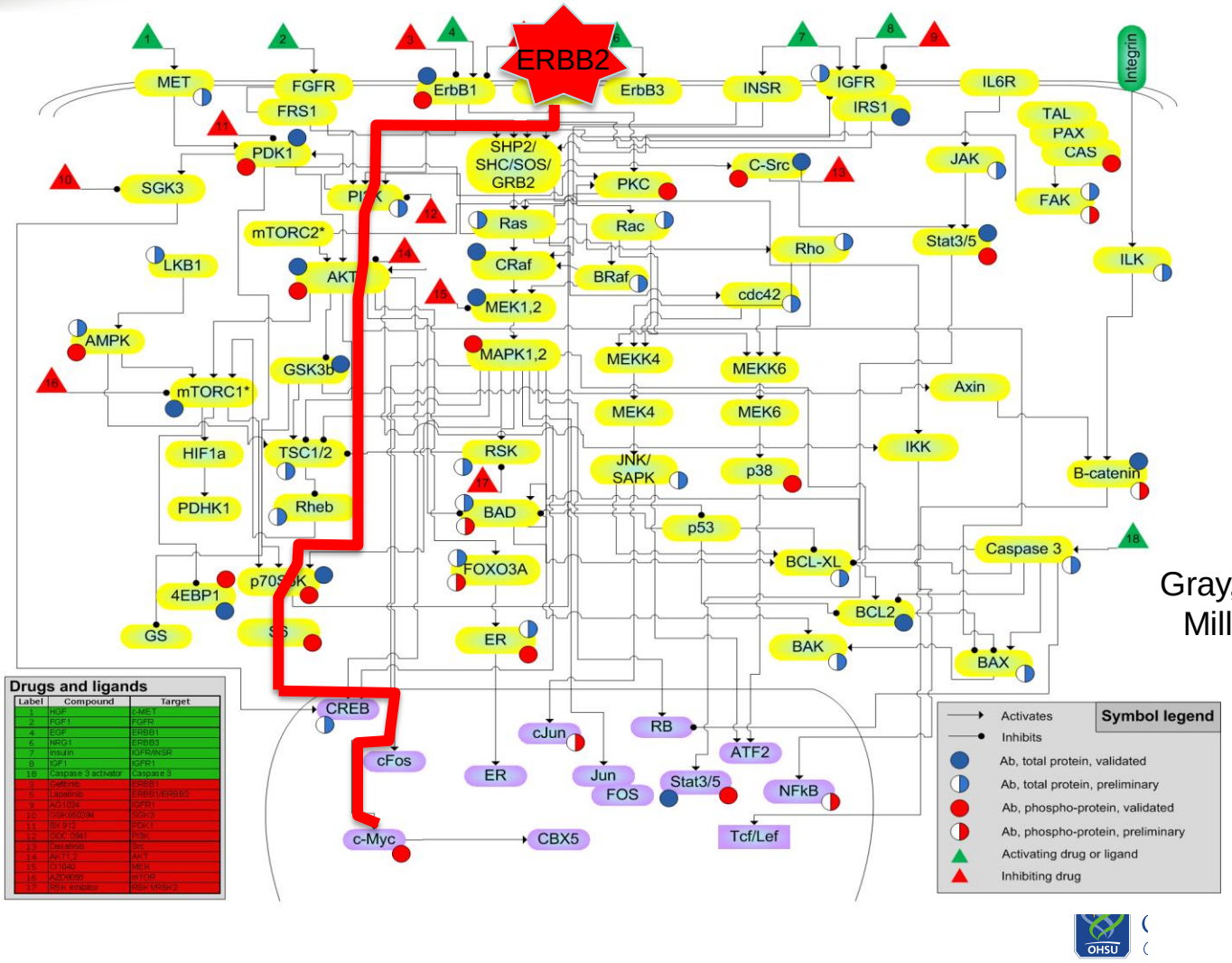




Di Leo et al., ASCO 2007

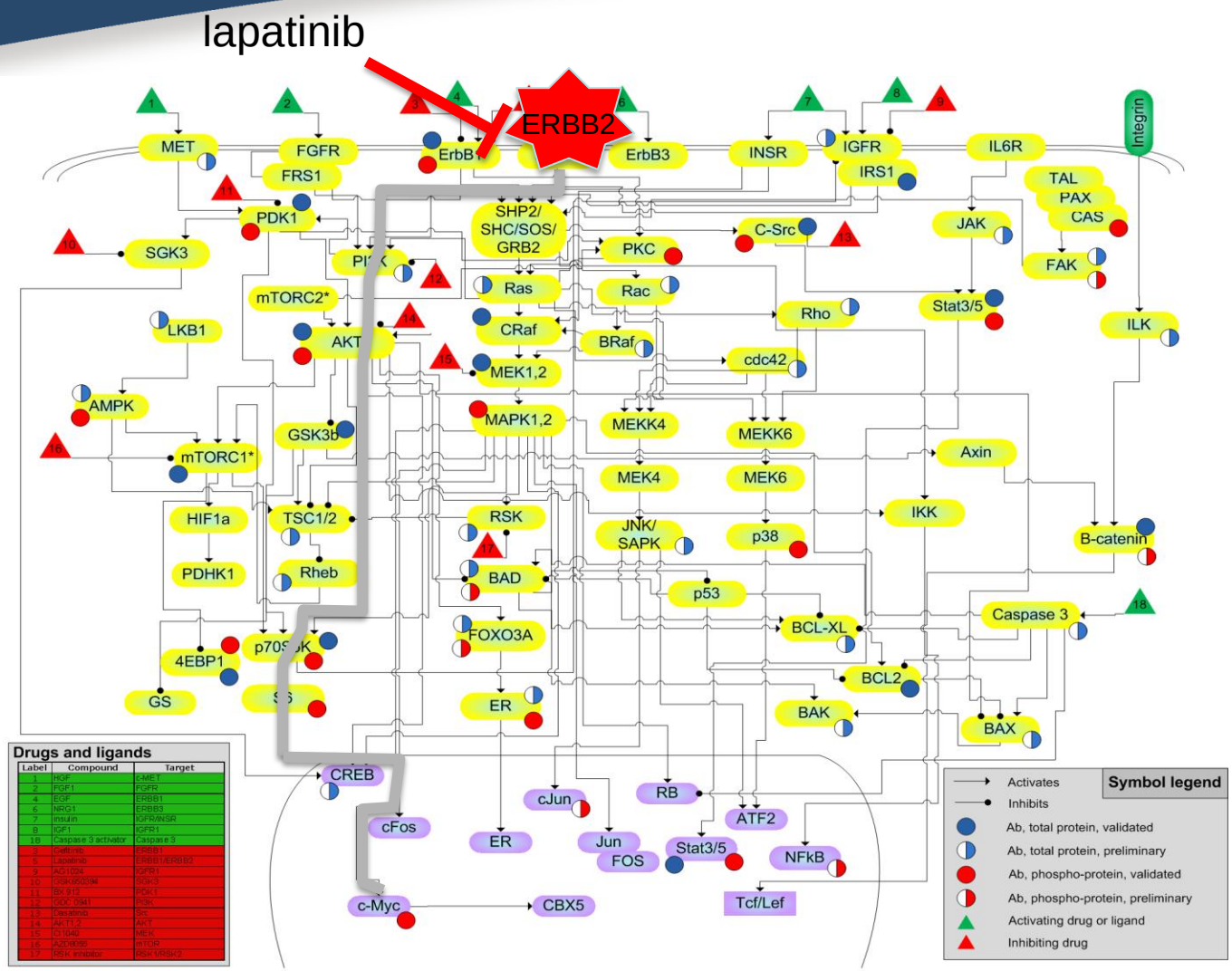
Development of strategies to target pathways deregulated by genome aberrations is a key goal

Understanding the larger signaling enterprise



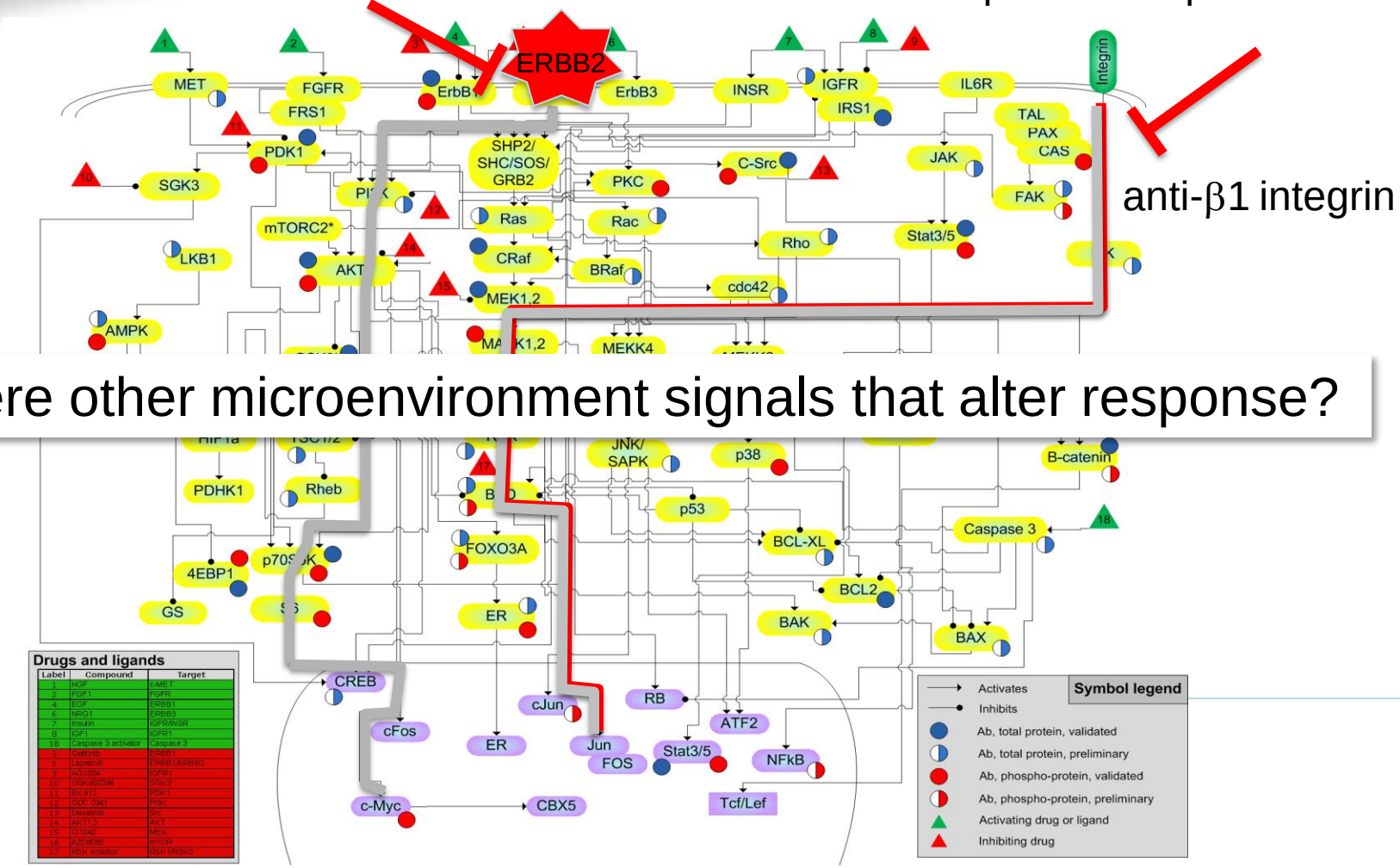
Gray, Spellman, Mills, Tomlin, Korn

Initial treatment blunts the aberration-driven activation



Microenvironment signaling can bypass inhibitors

Activation of β1 integrin signaling reduces response to lapatinib



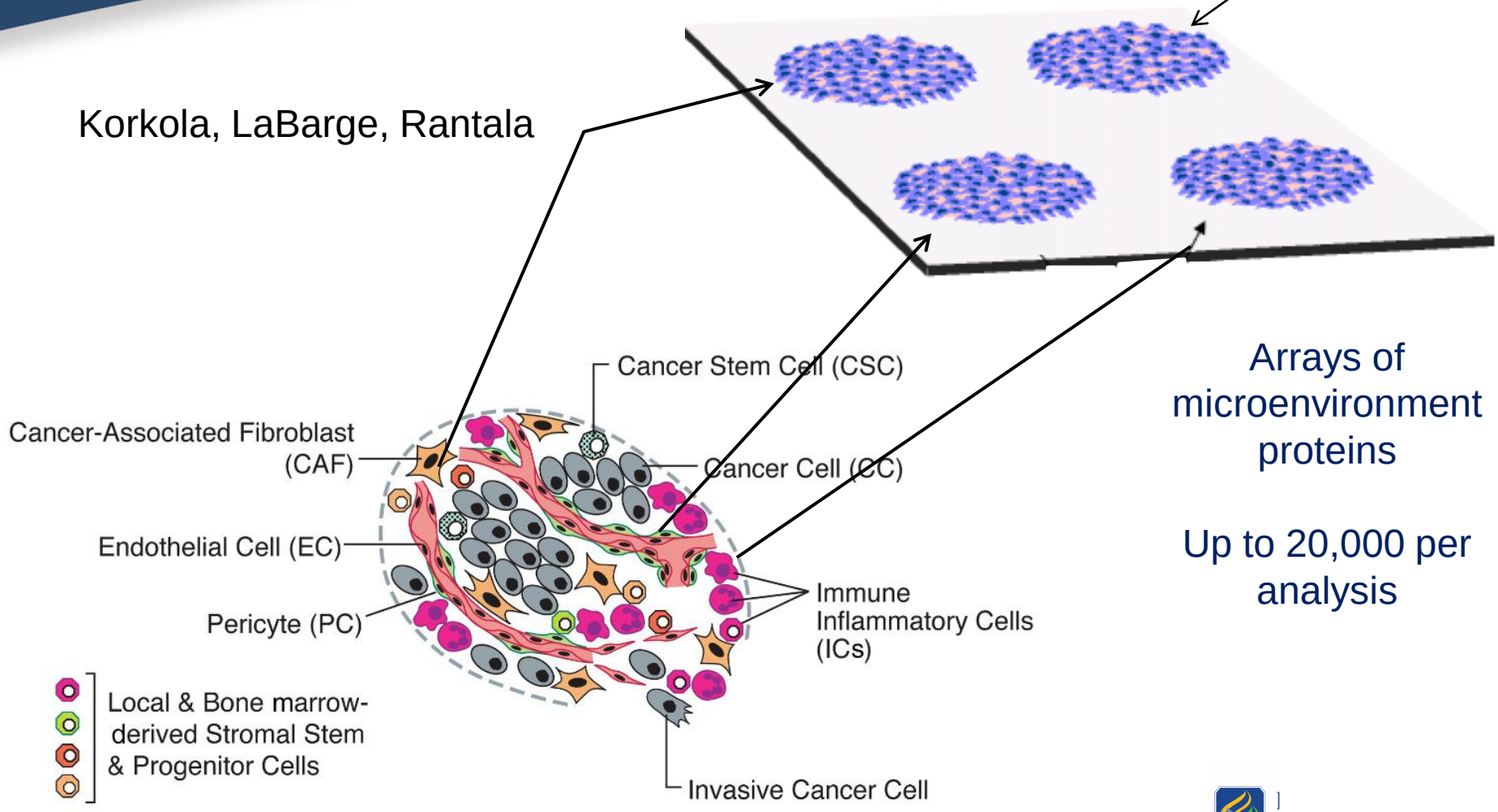
Are there other microenvironment signals that alter response?

Modeling the microenvironment

Microenvironment microarrays

Cancer and normal cells

Korkola, LaBarge, Rantala



Arrays of microenvironment proteins

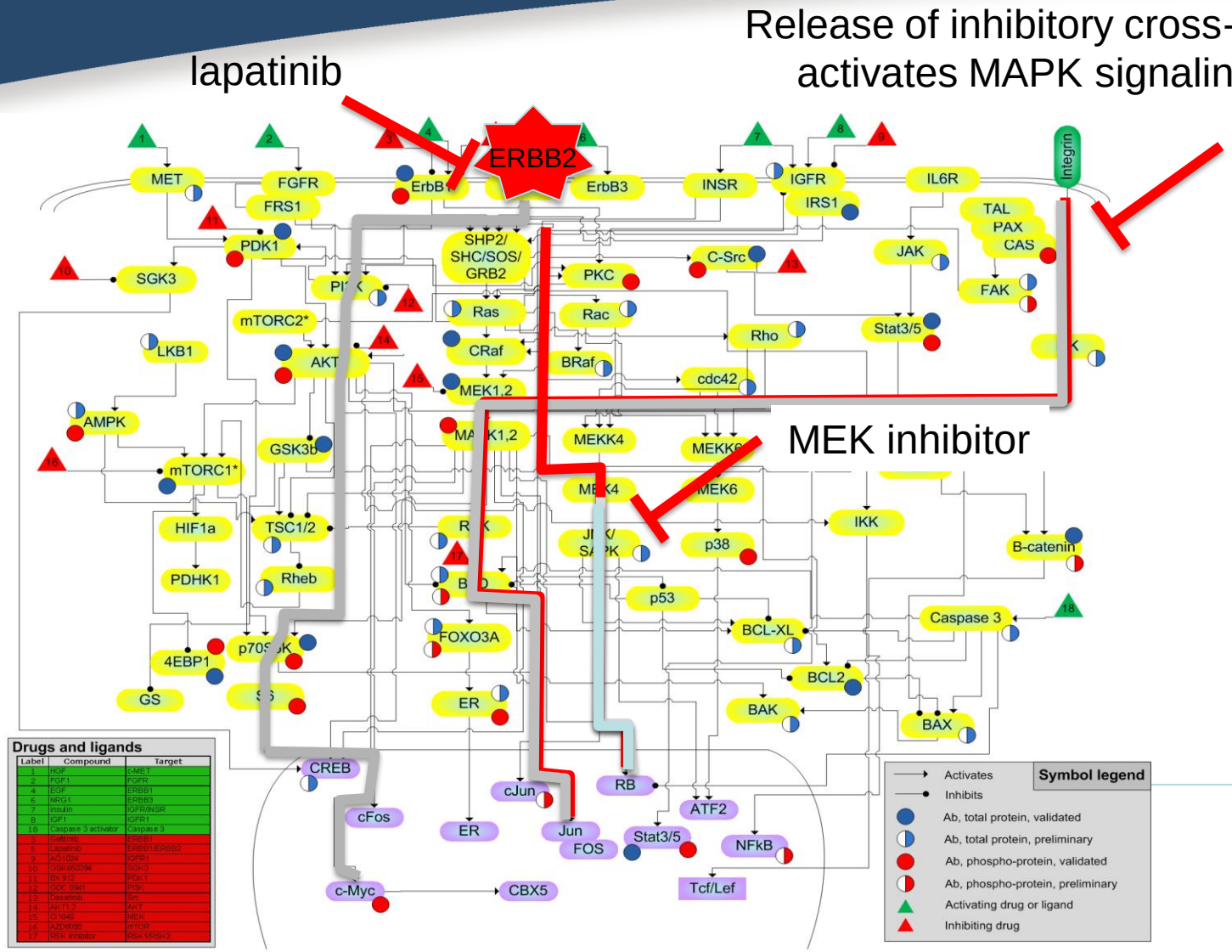
Up to 20,000 per analysis

Predicting responses to cancer therapies

Topics for discussion

- Linking genes/pathways to therapeutic response
- Understanding the role of the microenvironment
- Visualizing the nanoenvironment – exploring the mechanics of signaling

Activation of bypass pathways



Activation of bypass pathways

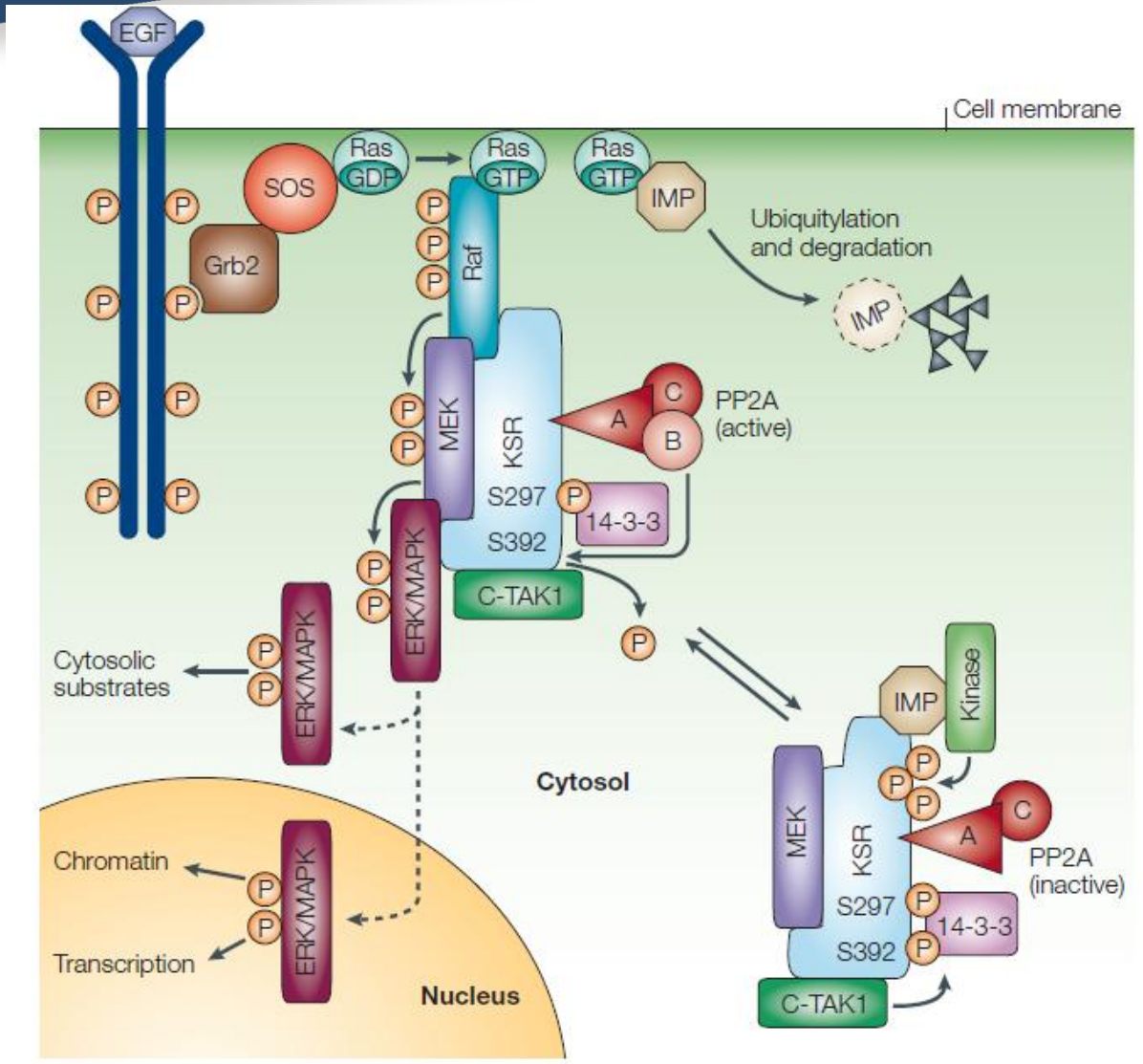
Combinatorial Rx becomes increasingly toxic unless targeting is very precise

How does all of this information get integrated?

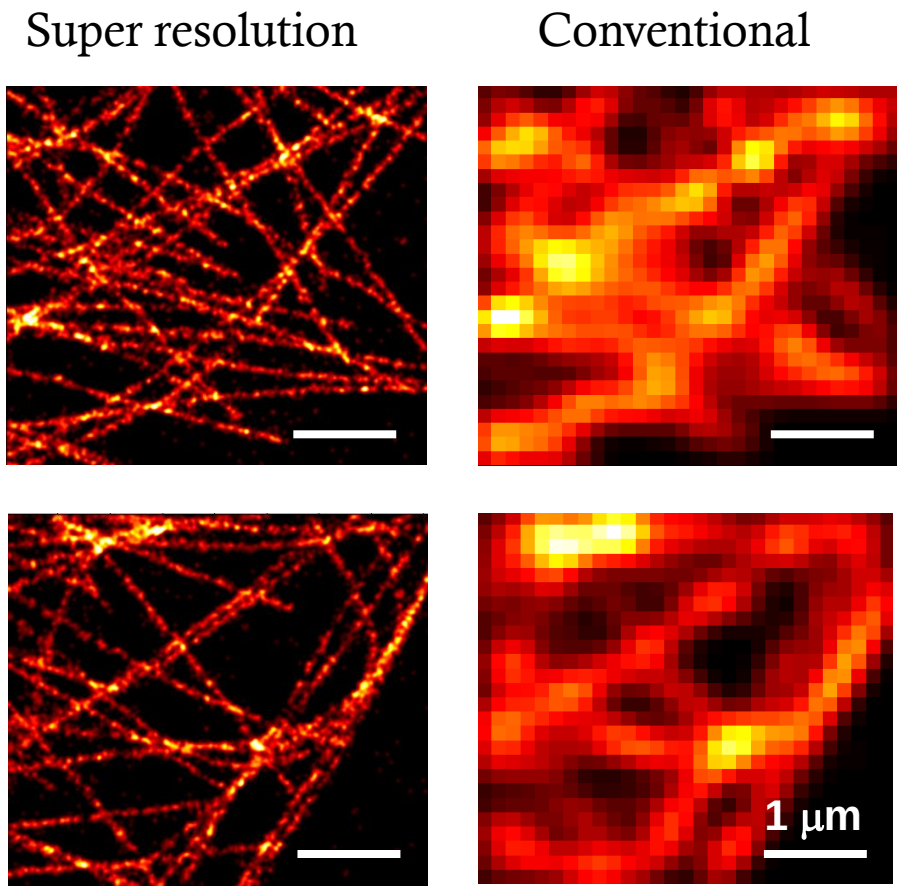
We don’t know where or how it gets translated into action (proliferation, death, etc)

Goal – visualizing the signaling architecture

An approach to assessment of digital signal transport



Advances in imaging, chemistry and computational Analysis can allow these structures to be seen



Fluorescence imaging at nanometer resolution

Super resolution fluorescence microscopy to the rescue

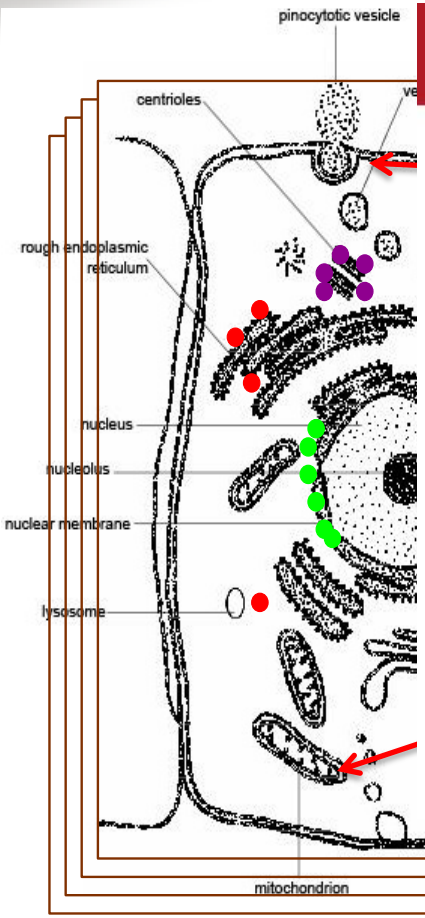
Super resolution imaging concept

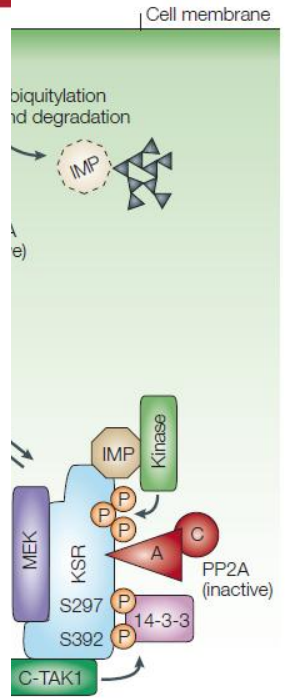
- The average X, Y location of a fluorescent molecule can be determined with few nanometer accuracy
- The locations of two closely spaced fluorescent molecules can be mapped with this precision as long as only one is on at a time
- Super resolution images are built up over time by switching on a few dye molecules, recording their positions, switching them off and doing a few more

Better imaging through chemistry

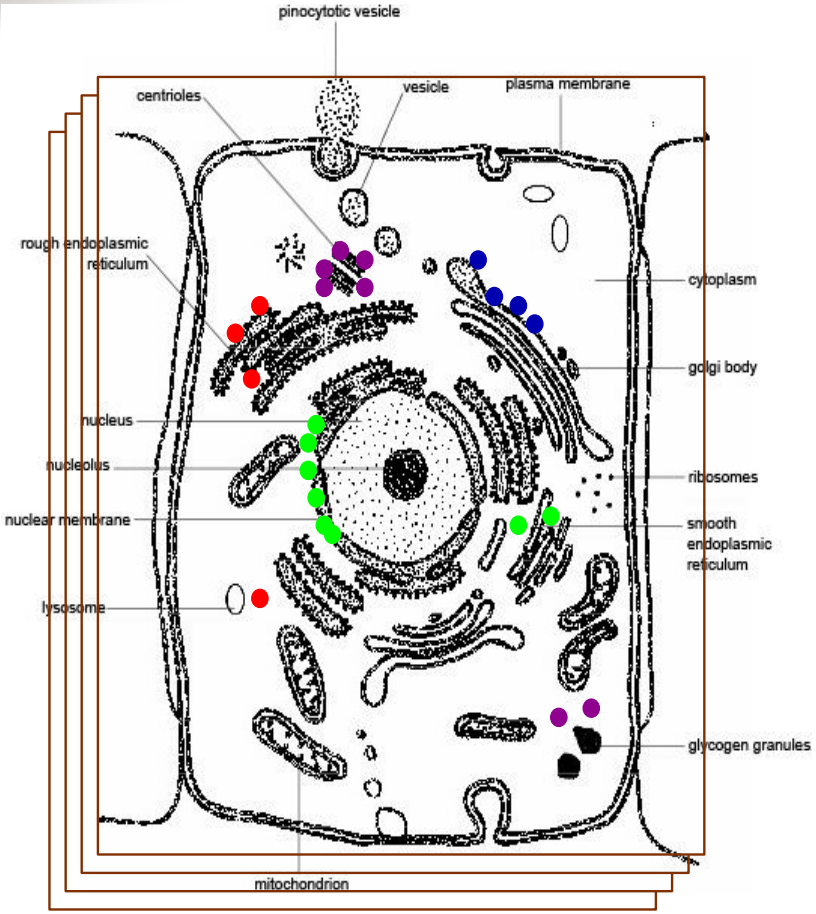
Identifying signal action centers

Localization of signaling structures defined using FM
on cellular action centers defined using EM





Modeling Paradigm: Encoding the State Space



Targets

- ERK/MAPK
- MEK
- RAF
- KSR

×

Structures

- Cell Membrane
- Cytosol
- Nuclear Membrane
- Nucleus
- Golgi Body
- Endoplasmic Reticulum
- Lysosomes

E.g., State Value (● ERK/MAPK, Golgi Body) = 5

Model Space |M| = O (|Targets| x |Structures|)

Cancer genomics and imaging

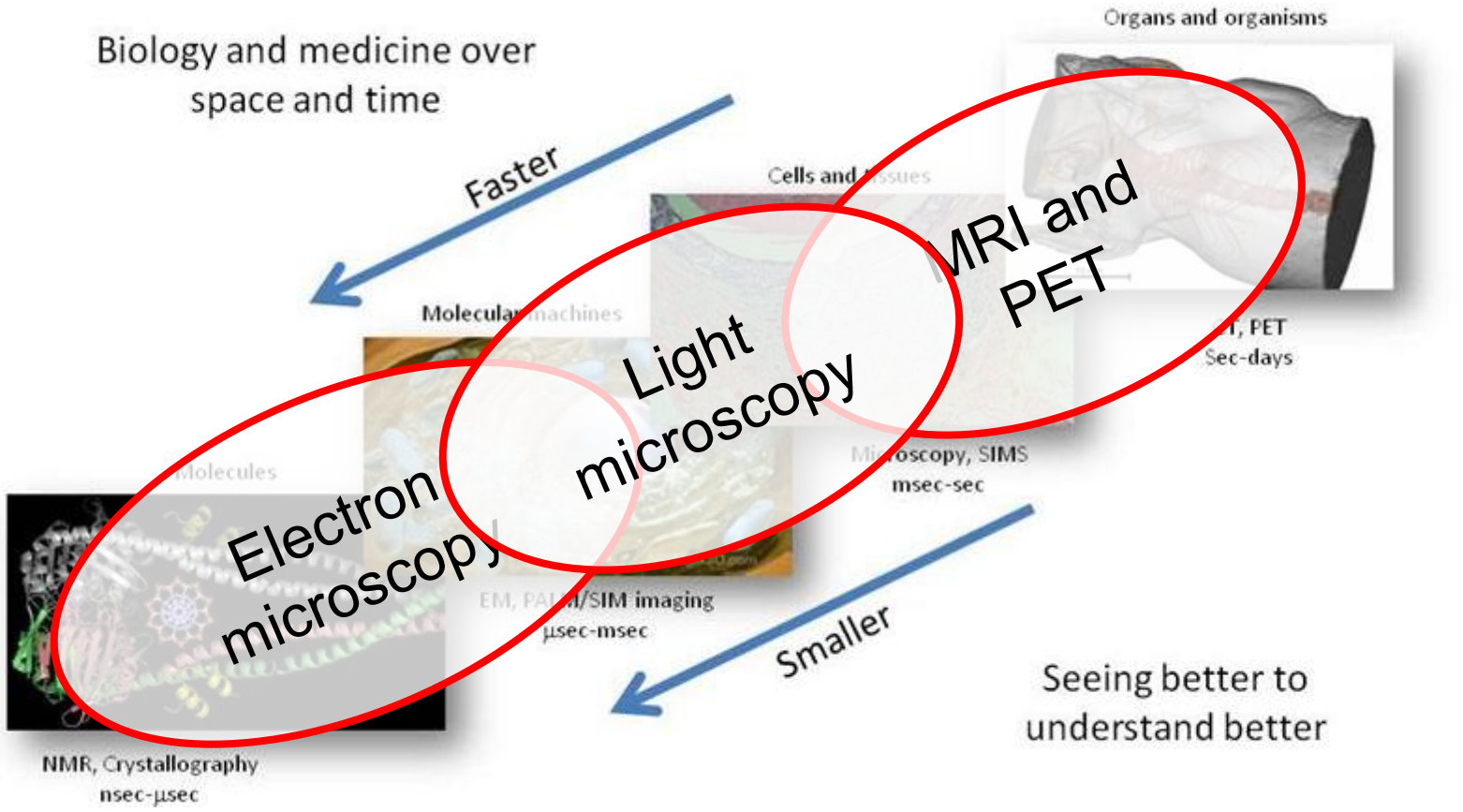
Aberration catalog will soon be completed for most major cancer types

High throughput, high content imaging strategies can define the impact of microenvironments on response

Imaging the micro- and nano-environments will facilitate development of more robust and precisely targeted therapies

Research pursued under the auspices of the OHSU Center for Spatial Systems Biomedicine

Integrating engineering, imaging, biology, chemistry and computer science to advance multiscale imaging science

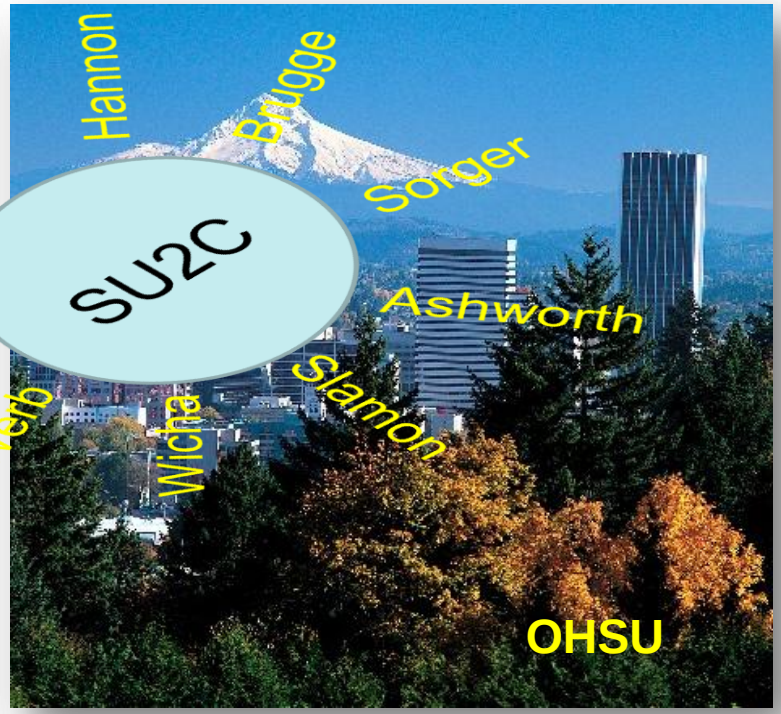


Omics and beyond

The collaborative village

SPORE, TCGA, DOD, PSOC, CPTAC, ICBP, I SPY

Lawrence Berkeley National Laboratory
University of California San Francisco



Oregon Health and Science University
Center for Spatial Systems Biomedicine

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NCI Center for Cancer Systems Biology, TCGA, DOD, Bay Area Breast Cancer SPORE, Atwater foundation, GSK, Roche, Millenium, Pfizer, Progen, Cytokinetics, Cell Biosciences, SU2C, FEI